Light and turgor affect the water permeability (aquaporins) of parenchyma cells in the midrib of leaves of Zea mays

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Received 15 August 2007; Revised 24 September 2007; Accepted 9 October 2007

Abstract
In response to light, water relation parameters (turgor, half-time of water exchange, $T_{1/2}$, and hydraulic conductivity, $L_p$; $T_{1/2}=1/L_p$) of individual cells of parenchyma sitting in the midrib of leaves of intact corn (Zea mays L.) plants were investigated using a cell pressure probe. Parenchyma cells were used as model cells for the leaf mesophyll, because they are close to photosynthetically active cells at the abaxial surface, and there are stomata at both the adaxial and abaxial sides. Turgor ranged from 0.2 to 1.0 MPa under laboratory light condition (40 $\mu$mol m$^{-2}$ s$^{-1}$ at the tissue level), and individual cells could be measured for up to 6 h avoiding the variability between cells. In accordance with earlier findings, there was a big variability in $T_{1/2}$s measured ranging from 0.5 s to 100 s, but the action of light on $T_{1/2}$s could nevertheless be worked out for cells having $T_{1/2}$s greater than 2 s. Increasing light intensity ranging from 100 $\mu$mol m$^{-2}$ s$^{-1}$ to 650 $\mu$mol m$^{-2}$ s$^{-1}$ decreased $T_{1/2}$ by a factor up to five within 10 min and increased $L_p$ (and aquaporin activity) by the same factor. In the presence of light, turgor decreased due to an increase in transpiration, and this tended to compensate or even overcompensated for the effect of light on $T_{1/2}$. For example, during prolonged illumination, cell turgor dropped from 0.2 to 1.0 MPa to –0.03 to –0.05 MPa. Overall, the effects of light on $T_{1/2}$ ($L_p$) were overriding those of turgor only when decreases in turgor were less than about 0.2 MPa. Otherwise, turgor became the dominant factor. The results indicate that the hydraulic conductivity increased with increasing light intensity tending to improve the water status of the shoot. However, when transpiration induced by light tends to cause a low turgidity of the tissue, cell $L_p$ was reduced. It is concluded that, when measuring the overall hydraulic conductivity of leaves, both the effects of light and turgor should be considered. Although the mechanism(s) of how light and turgor influence the cell $L_p$ is still missing, it most likely involves the gating of aquaporins by both parameters.

Key words: Aquaporins, cell pressure probe, hydraulic conductivity, leaf, light, parenchyma cells, turgor, Zea mays.

Introduction
There is a lot of evidence that the overall hydraulic conductance of leaves ($K_{leaf}$) is substantially affected by light, but as yet the mechanisms of changes in $K_{leaf}$ are poorly understood (Nardini et al., 2005; Sack and Holbrook, 2006; Cochard et al., 2007). For example, Lo Gullo et al. (2005) showed that leaf conductance was positively correlated with photosynthetically active radiation (PAR) in evergreen and deciduous trees. In laboratory experiments, Sack et al. (2002) demonstrated that $K_{leaf}$ of Quercus leaves measured by a high pressure flow meter (HPFM) was bigger under irradiance (>$1200 \mu$mol m$^{-2}$ s$^{-1}$) than that measured under ambient light condition. Using the HPFM technique, Tyree et al. (2005) observed similar phenomena in six tree species. However, Brodribb and Holbrook (2004) showed midday depressions of $K_{leaf}$ and stomatal conductance of a tropical tree, when the water status was unfavourable.
Although the mechanism(s) of responses of \( K_{\text{leaf}} \) to irradiance are not yet clear, Tyree et al. (2005) used the HPFM to find out why \( K_{\text{leaf}} \) increased during short-term illumination of 30 min. They excluded a contribution of the stomatal conductance and suspected that the increase in \( K_{\text{leaf}} \) was due either to changes in the vascular component by hydrogel effects (Zwieniecki et al., 2001), or to changes in non-vascular components, probably related to water channels (aquaporins, AQPs). There is increasing evidence that AQPs play a key role in plant water relations (Steudle and Henzler, 1995; Maurel, 1997; Kjellbom et al., 1999; Tyerman et al., 1999; Steudle, 2000, 2001; Maurel and Chrisepeels, 2001; Javot and Maurel, 2002). Cell water permeability may increase either by de novo expression of AQPs or by an opening of closed channels (‘gating’). In roots of Lotus japonicus and in leaves of Samaens saman, the diurnal changes in hydraulic conductance have been attributed to changes in levels of mRNA encoding for AQPs (Henzler et al., 1999; Moshelion et al., 2002). In leaves of walnut, the increase in \( K_{\text{leaf}} \) by light was in accordance with the transcript abundance of two aquaporins, and this effect occurred within 30 min (Cochard et al., 2007). Aside from the regulation at transcript levels, 30-min-light treatments causing an increase of \( K_{\text{leaf}} \) are likely to involve the action of AQPs tending to open in response to light treatment (Nardini et al., 2005; Tyree et al., 2005). However, there have been as yet no direct measurements of changes of the cell hydraulic conductivity caused by irradiance (i.e. by a gating of AQPs by light).

Using overall measurements, there are difficulties in confining the components affected by light. Overall leaf hydraulic conductivity in kg H\(_2\)O m\(^{-2}\) s\(^{-1}\) MPa\(^{-1}\), or its inverse, the resistivity, has different components. Components are arranged either in series or in parallel such as the resistances of petioles, leaf lamina (consisting of living cells and vascular components), or stomata. At steady-state, resistances form a complicated network, and the regulation of individual conductances (resistances) of different components will result in overall changes of leaf hydraulics. In recent studies, it has been proposed that up to 90% of \( K_{\text{leaf}} \) may be attributed to living tissue (Cochard et al., 2004; Nardini et al., 2005; Sack et al., 2005). As living cells can effectively regulate their water permeability by the expression or gating of AQPs, living cells could substantially contribute to \( K_{\text{leaf}} \). However, the problem with the figures from overall measurements is the reliable quantification of the components that eventually determine \( K_{\text{leaf}} \). This conceptual work is still missing. Another item that should be considered in the overall measurements is whether or not experimental conditions are similar to the real. Serious doubts have been raised as to whether or not leaf conductances measured with the HPFM technique relate to the real situation (Nardini et al., 2005). With the HPFM, leaf tissue is usually infiltrated with pressurized water via the xylem, filling intercellular spaces so that liquid water is eventually dropping out of stomata or hydathodes. This is not the situation in a transpiring leaf. However, people using the HPFM tended to convince themselves and others that what they measured was meaningful, namely, by comparing their data with those obtained by other techniques (Sack et al., 2002; Nardini et al., 2005). Brodribb and Holbrook (2006) showed that \( K_{\text{leaf}} \) would be different, when measured either in the steady-state (evaporation technique) or by following transient water uptake (capacitive recharge, relaxation of water potential upon rehydration through the petiole) and discussed that the former method would be a more realistic measure than the latter. These authors discussed the discrepancy in terms of decreases of turgor pressure, which were substantial and immediate, when the evaporation technique was used to measure \( K_{\text{leaf}} \). This may indicate an effect of turgor on \( K_{\text{leaf}} \).

In the present study, it was intended to fill the gap between overall measurements of leaf hydraulics and the molecular level, i.e. direct action of AQPs. A cell pressure probe (CPP) was used to do so. At present, the CPP is the most sensitive technique used for measurements of water permeability of intact cells (half-time of water exchange, \( T_{1/2} \) and hydraulic conductivity, \( L_p; T_{1/2} \leq 1/L_p \). It should be emphasized that, in this first study, it was not intended to identify mechanisms of the gating of AQPs in leaves in response to light. Rather, using a CPP, an examination was made as to how the permeability of individual cells in the leaves of intact corn plants changed in response to light and whether or not these changes would correlate to what was found by others at the whole leaf level. The leaf tissue used for the measurements was the midrib of 4–8-week-old corn plants, where cells close to vascular bundles could be investigated in response to increasing light intensity (ambient light intensity = 40 \( \mu \)mol m\(^{-2}\) s\(^{-1}\), light treatments = 100, 160, or 650 \( \mu \)mol m\(^{-2}\) s\(^{-1}\)). In the past, the parenchyma cells in the midrib tissue have been shown to be an excellent object for measuring turgor and hydraulic conductivities of individual cells over periods of time of up to 6 h. This is a prerequisite for the studies, because it is known that cells from higher plant tissue may substantially vary their \( T_{1/2} \) or turgor (Tomos et al., 1981; Westgate and Steudle, 1985; Nonami et al., 1987; Zhu and Steudle, 1991). Measuring effects on individual cells eliminates the variability between cells. Due to their big size, cells were easy to puncture. As they were located in the vicinity of photosynthetically active cells, stomata, and xylem vessels, parenchyma cells from the midrib may be considered as a good model for mesophyll cells from the leaf blade, which are much more difficult to measure over long terms (Frensch and Schulze, 1988).

During the studies, it turned out that, besides light, cell turgor was a variable as well, which may affect cell \( L_p \).
Hence, the effects of the two variables had to be separated using a root-pressure chamber to keep the turgor pressure of individual cells constant while varying light intensity. This could not be done in the overall studies mentioned above. The results clearly showed that there were separate effects of light and turgor on $T_{1/2} (Lp)$.

Materials and methods

Plant material

Corn (Zea mays L. cv. monitor) plants were grown in a greenhouse of Bayreuth University from caryopses in soil in plastic pots (1.7 l; diameter: 140 mm; depth: 110 mm). Plants were watered daily. Experiments were conducted on 4–8-week-old plants that were 0.8–1.2 m tall and had about eight leaves. A maize plant was brought from the greenhouse, and the experimental set-up was used as in Wei et al. (1999). Fourth or fifth leaves of the plants were used counting from the oldest. Leaf blades were 0.6–1 m long. All measurements were made on cells in the midrib region located 100–200 mm behind the tip of leaves. At this point, the midrib looked like a half cylinder with a diameter of about 1 mm (Fig. 1A, B). At its convex surface, it contained several parallel vascular bundles, one rather large located in the centre of the periphery with about five bundles on both sides. Within the centred large bundle, there were two metaxylem vessels, each about 50 µm in diameter ($v$). There was a group of small tracheary elements between them, and protoxylem elements or protoxylem lacunae in the direction to the adaxial surface. Within the smaller bundles, vessel diameters were around 20 µm. Between bundles, there were stomata at the abaxial side (about 100/mm²; Fig. 1C). There were also stomata on the adaxial side of midribs (about 45/mm²; Fig. 1D). The bulk of the midrib tissue consisted of about five layers of parenchyma cells containing no chlorophyll. These cells were used in the experiments. The position of parenchyma cells used was estimated from the depth of microcapillary tip inside the midrib tissue. The depth was 100–250 µm from the abaxial surface of the midrib (and stomata). The cells used were usually of the second layer of parenchyma cells, when counting from the abaxial surface. They were about 100 µm away from the vessels and 50 µm away from photosynthetically active tissue.

Experimental set-up using a CPP

The cell pressure probe was mounted on a Leitz manipulator (Wetzler, Germany) that was screwed onto a thick iron plate and placed on a heavy stone table. Using magnetic bars, an intact leaf was mounted upside down on a metal sledge to expose the midrib for measurements of cell hydraulics in a secure way. Cells in the midrib were punctured using the microcapillary of a CPP, which was filled with silicon oil up to the $\approx 8$ µm tip (oil type AS4 from Wacker, Munich, Germany). When cells were punctured, a meniscus formed within the tip between the cell sap and the oil. With the aid of the probe, the meniscus was gently pushed back close to the surface of the midrib to restore the cell sap volume close to its original value. Turgor pressure ($P$) was measured by a pressure transducer and recorded by a computer. The function of the CPP has been described in many earlier papers (Steudle, 1993; Henzler and Steudle, 1995). To investigate the hydraulic conductivity of cell membranes ($Lp$), hydrostatic relaxations of turgor pressure were induced with peak pressure differences being less than 0.1 MPa to avoid possible effects by big pressure pulses (‘energy-injection’; Wan et al., 2004). From half-times of hydrostatic relaxation ($T_{1/2}$), $Lp$ was calculated by:

$$Lp = \frac{\text{constant}}{T_{1/2}}$$

Fig. 1. Cross-sections and surface patterns of the midrib of a corn leaf. Red triangles indicate the centre of the midrib. (A) Section was taken 150 mm from the tip of leaf number four of an 8-week-old plant (counting from the oldest one). The midrib looked like a half cylinder having a diameter of 1 mm. The bulk of the midrib tissue consisted of about five layers of parenchyma cells and at the periphery there was one big vein in the centre and several smaller veins. Bar=100 µm. (B) Part of (A) showing that the big vein has two large metaxylem vessels ($v$). Water relation parameters were determined using a CPP for cells, which were usually in the second layer of parenchyma cells, counting from the abaxial surface. It be can seen from the figure that there was some variability in the diameters of the cells (see Table 1). (C) Imprint (obtained with nail polish) of the abaxial side of the leaf. The abaxial side had about 100 stomata mm⁻² leaf surface. Bar=100 µm. (D) The surface replica of adaxial side showed about 45 stomata per mm² of leaf surface. Bar=100 µm. Arrows indicate guard cells.
\[ Lp = \frac{V}{A} \times \ln(2) T_{1/2}(\varepsilon + \pi^t) \]

Here, the cell geometry (\(V\), volume, \(A\), surface area) was measured from cross-sections (Fig. 1) and longitudinal sections (not shown in Fig. 1) to work out the mean values of \(V\) and \(A\), and \(\pi^t\) is the osmotic pressure of a cell and could be approximated by the turgor pressure measured when transpiration was low under laboratory light conditions. The volumetric elastic modulus of the cell (\(\varepsilon\)) was determined by the change in pressure (\(dP\)) according to the relative volume change (\(dV/V\)) by:

\[ \varepsilon = V \frac{dP}{dV} \approx V \frac{\Delta P}{\Delta V} \]

As summarized in Table 1, the mean \(\varepsilon\) was calculated as 3.8±3.4 MPa (range: 0.4–13 MPa), incorporating the cell volume. Volumes of cells were calculated assuming that they were cylindrical. The mean cell volume was 2.4×10^{-13} m^3 (240 pl) with a standard deviation of ±1.2×10^{-13} m^3 (±120 pl or ±50%; Table 1, error propagation considered). Anatomical data and elastic moduli were similar to those given by Westgate and Steudle (1985), who used the same tissue of younger plants of an age of 13–17 d. Usually, \(\varepsilon\) was much bigger than \(\pi^t\). In most of the cases, \(T_{1/2}\) was used as a direct measure of change in cell \(Lp\), as \(\varepsilon\) did not change significantly during measurements with individual cells.

**Illumination experiment**

In the laboratory, where the experiments were performed, the light intensity was 5 μmol m^{-2} s^{-1} (temperature=20–30 °C; RH=30–60%). In order to run the CPP, an Osram halogen lamp (150 W, Xenon phot HLX, Munich, Germany) was used through glass fibre optics (Schott, Mainz, Germany) to illuminate the microcapillary and tissue near the cell punctured at a light intensity of about 40 μmol m^{-2} s^{-1}. After a cell was punctured, half-times from hydrostatic relaxations, \(T_{1/2}s\), were measured continuously. When for 10 min cells had stable \(T_{1/2s}\), light was switched on. Using a 400 W mercury vapour lamp (Siemens AG, Frankfurt, Germany), the light intensity at the leaf level was then changed to different levels.

### Table 1. Dimensions, turgor pressure (P), volumetric elastic modulus (\(\varepsilon\)), half time (\(T_{1/2}\)), and hydraulic conductivity (\(Lp\)) of leaf parenchyma cells of the second layer counting from the abaxial surface at a distance of 100–200 mm from the leaf tip (means ±SD)

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Value</th>
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<tbody>
<tr>
<td>Cell dimensions</td>
<td>Diameter (μm) 63±19 (n=59 cells, 3 plants)</td>
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<tr>
<td></td>
<td>Length (μm) 76±20 (n=56 cells, 3 plants)</td>
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<tr>
<td></td>
<td>Volume (10^{-13} m^3) 2.4±1.2</td>
</tr>
<tr>
<td>Water relations parameters</td>
<td>Stationary turgor pressure (P; MPa) 0.2–1.0, 0.5±0.2 (n=74 cells)</td>
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<td></td>
<td>Volumetric elastic modulus ((\varepsilon); MPa) 0.4–13, 3.8±3.4 (n=41 cells)</td>
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<td></td>
<td>((\varepsilon)/V; 10^{13} MPa m^{-3}) 1.6±1.2</td>
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<tr>
<td></td>
<td>Half-time of water exchange ((T_{1/2}); s) 0.5–95 (n=74 cells)</td>
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<tr>
<td></td>
<td>Hydraulic conductivity ((Lp); 10^{-6} m s^{-1} MPa^{-1}) 5.1–0.026</td>
</tr>
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As summarized in Table 1, the mean \(Lp\) was calculated as 3.8±3.4 MPa (range: 0.4–13 MPa), incorporating the cell volume. Volumes of cells were calculated assuming that they were cylindrical. The average cell volume was 2.4×10^{-13} m^3 (240 pl) with a standard deviation of ±1.2×10^{-13} m^3 (±120 pl or ±50%; Table 1, error propagation considered). Anatomical data and elastic moduli were similar to those given by Westgate and Steudle (1985), who used the same tissue of younger plants of an age of 13–17 d. Usually, \(\varepsilon\) was much bigger than \(\pi^t\). In most of the cases, \(T_{1/2}\) was used as a direct measure of change in cell \(Lp\), as \(\varepsilon\) did not change significantly during measurements with individual cells.

**Results**

**Cell turgor pressure of parenchyma cells and its response to light regimes**

After introducing the tip of the microcapillary of a CPP into a midrib parenchyma cell, turgor pressure either became stable within a few minutes, or there was a transient overshoot in turgor due to the fact that the meniscus had to be pushed close to the surface of the leaf to restore cell volume close to the original. In any case, turgor pressure stabilized within 30 min and was measured stable for 6 h at maximum. When cells were leaky, turgor continuously dropped, and these cells were disregarded. In Table 1, turgor pressure values are given for 74 non-leaky cells, which ranged between 0.2 MPa and 1.0 MPa under laboratory light conditions (40 μmol m^{-2} s^{-1} at the tissue level). It can be seen from Table 1 that there was some scatter in the absolute values of turgor, half-time, elastic modulus, and cell \(Lp\), which has been known from earlier studies of plant tissues such as the root cortex and leaf epidermis and mesophyll (Toms et al., 1981; Westgate and Steudle, 1985; Nonami et al., 1987; Zhu and Steudle, 1991).

Following an increase in irradiance, turgor pressure decreased in response to increased transpiration. A representative response in turgor of a midrib cell to short-term (18 min) illumination is shown in Fig. 2A (light intensity of 100 μmol m^{-2} s^{-1}). According to the figure, there was a delay of about 5 min in the response of turgor,

by changing the height of the lamp. Following light treatments, measurements of relaxations (\(Lp\)) were continued for up to 1.5 h. During illumination, the lamp was set up above a plant and the plant experienced a gradient in light intensity. The upper part of the plant experienced a somewhat higher light intensity, and light intensity was measured at the point of CPP measurements. Light intensities at the level of a given leaf were varied at stages of 100, 160, or 650 μmol m^{-2} s^{-1} PAR.

**Illumination experiments at constant turgor and using a pressure chamber**

In order to separate the effects of turgor from those of light, a pressure chamber encasing the root was used to keep turgor constant during illumination of 160 μmol m^{-2} s^{-1}. The pot containing the root was sealed in a metal pressure chamber using rubber seals (Wei et al., 1999). Roots were pressurized with pressurized air from a gas tank. Measurements of relaxations (\(Lp\)) were continued during light phases.

**Measurement of rates of transpiration by a porometer**

Following CPP measurements, a steady-state porometer (LI-1600 from Li-Cor, Lincoln, Nebraska, USA) was used to measure transpiration (\(E\) in mmol m^{-2} s^{-1}), relative humidity (RH in %), and air temperature near the area where CPP measurements were performed. After 1 h from the CPP measurements, porometer measurements were conducted using the same time of exposure to light as during the CPP measurements.

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which was due to the time required for opening stomata. When light was switched off, both turgor and transpiration required a time delay of about 10 min to assume their previous values. For the cell given in Fig. 2A, the half-time of recovery of turgor ($t_{1/2} = 400$ s, when fitted exponentially) was similar to that of the decrease in transpiration ($t_{1/2} = 390$ s) suggesting that transpirational water losses rate-limited changes in turgor. The original turgor pressure was re-attained after about 20 min. During the period of illumination, the surroundings of the measured tissue experienced an increase in temperature of 1.2 °C and a decrease of RH of 5 %, respectively. The increase in temperature at the leaf surface was 1.4 °C.

Figure 2B shows a typical response of a midrib cell, which reduced turgor to a steady value, following an illumination with a duration of 30 min at a light intensity of 160 μmol m$^{-2}$ s$^{-1}$. Turgor declined to a steady minimum value of close to zero ($t_{1/2} = 240$ s). During light off, there was a somewhat slower recovery of turgor ($t_{1/2} = 440$ s). Usually, the time required for recovery was bigger than that for turgor loss during illumination. As seen in Fig. 2B, the minimum steady values of turgor were often close to zero (atmospheric pressure). The same type of recovery was found, even when turgor dropped to pressures of −0.03 MPa (below atmospheric).

**Half-times of water exchange ($T_{1/2}$) of parenchyma cells as measured by CPP**

After introducing the tip of the microcapillary of the CPP into a midrib parenchyma cell, half-times of hydrostatic relaxations of turgor pressure ($T_{1/2}$) were measured for up to 6 h (on average, for 3 h). The measured $T_{1/2}$ in 74 cells showed a big range of values of between 0.5 s and 95 s (Table 1; corresponding $L_p$ values for the smallest and largest half times were $5.1 \times 10^{-6}$ and $2.6 \times 10^{-5}$ m s$^{-1}$ MPa$^{-1}$, respectively). Different from earlier studies on cortical cells of roots of young corn plants (Wan et al., 2004), there was no transient increase in $T_{1/2}$ after puncturing, which may have indicated a transient closure of AQPs. Rather, the $T_{1/2}$s of leaf cells varied somewhat with time even in the absence of any treatment, but were stable during periods of 10–20 min as verified from the long-term measurements with individual cells (see Discussion). Hence, when measuring the effects of light and turgor, we concentrated on these time periods. In this way, it was possible to get rid of the ‘noise’ in $T_{1/2}$. In order to get rid of variations between cells, for statistics changes in $T_{1/2}$ were given as relative rather than absolute values. The half-time before illumination was taken as the control half-time, $T_{1/2}^c$.

**Effects of light on $T_{1/2}$ of parenchyma cells**

**Effects of light on $T_{1/2}$ at constant turgor:** During illumination, the effect of light could have been masked by the
effect of turgor, which may have resulted in an increase of $T_{1/2}$ as turgor decreased. Therefore, turgor was kept constant using a root-pressure chamber solely to refer to the effects of light. As seen in Fig. 3A, there was an effect of light, which was independent of that of turgor. When keeping turgor constant, $T_{1/2}$ decreased by a factor of 3 within 30 min at a light intensity of 160 μmol m$^{-2}$ s$^{-1}$ (increase of cell $L_p$; Equation 1). The results of this type of experiment are summarized in Fig. 4 (see below).

Effects of changes in turgor on $T_{1/2}$: According to Fig. 2, light of 100 and 160 μmol m$^{-2}$ s$^{-1}$ caused substantial decreases in turgor of bigger than 0.2 MPa. The latter could have affected $T_{1/2}$, in addition to the light. As seen in Fig. 3B, during longer periods of illumination at a light intensity of 160 μmol m$^{-2}$ s$^{-1}$, $T_{1/2}$ first decreased. However, at the low turgor, $T_{1/2}$ increased in spite of illumination, i.e. the effect of turgor was overriding that of light. The turgor increased again without pressurizing the root, and this sometimes happened, perhaps by an improved water supply from the root in response to a demand from the shoot (variable root hydraulics; Kramer and Boyer, 1995; Steudle and Peterson, 1998). Transient changes of turgor in response to light showing undershoots have been also observed by French and Schulze (1988) in their study on the effects of light on turgor of individual mesophyll and epidermis cells of leaves of Tradescantia virginiana. In the present study, when turgor pressure increased back to the original value using the root-pressure chamber, the $T_{1/2}$ recovered to the original during a light phase (Fig. 3B). Changes of $T_{1/2}$ should have reflected changes in cell $L_p$ rather than changes in the elastic modulus of cells ($\epsilon$; Equation 1), because $\epsilon$ remained constant during these experiments with individual cells (data not shown).

Overall, the experiments shown in Fig. 3 indicated that $T_{1/2}$ decreased by light, but there was an effect of turgor as well (see Discussion). Results of experiments such as those shown in Fig. 3 are summarized in Fig. 4. Here, the responses of $T_{1/2}$ to light of 160 μmol m$^{-2}$ s$^{-1}$ were compared either at constant turgor or at changing turgor. When turgor was kept constant within $+ 0.05$ MPa, light significantly reduced $T_{1/2}$ by a factor of 2.2 within 10 min and of 2.6 within 30 min (statistics with cells of $T_{c1}^2 > 2$ s; $n = 6$ cells). However, in the presence of a decrease in turgor greater than 0.2 MPa, $T_{1/2}$ first reduced, but when turgor had changed, usually when reaching at the minimum turgor, there were re-increases in $T_{1/2}$ by a factor

![Fig. 3.](https://academic.oup.com/jxb/article-abstract/58/15-16/4119/449291) Illumination reduced the half-time of water exchange, $T_{1/2}$, at constant turgor, but increased $T_{1/2}$ when turgor declined. (A) While turgor was kept constant at 0.55 MPa using a root-pressure chamber, a light intensity of 160 μmol m$^{-2}$ s$^{-1}$ caused a decrease in $T_{1/2}$ by a factor of about 3 in 30 min and $L_p$ increased by the same factor. (B) During illumination of 160 μmol m$^{-2}$ s$^{-1}$, $T_{1/2}$ first decreased, but increased again as turgor pressure decreased to a low value of 0.2 MPa. There was an increase in turgor during illumination and it caused an ameliorative effect on $T_{1/2}$. When turgor pressure was increased back to the original using the root-pressure chamber during a light phase, there was the same effect on $T_{1/2}$. Time zero was the time when cells were punctured. The peaks appearing in the turgor curve are hydrostatic relaxations.
of as large as 4 compared with $T_{1/2}^{c}$ (n=6–11 cells). Depending on the individual cells, the re-increase of $T_{1/2}$ happened between 10–25 min after the light was turned on. When turgor was increased back under the irradiance, $T_{1/2}$ recovered to the value as small as 75% of $T_{1/2}^{c}$ (n=4 cells).

Effect of light intensity on $T_{1/2}$: When cells with half-times of between 0.5 s and 2 s were exposed to light, there was hardly any response in $T_{1/2}$ within 10 min. This may be due to the fact that these cells already had their maximum cell $Lp$ (maximum activity of AQPs; see Discussion). For cells having $T_{1/2}^{c}$s of larger than 2 s, illumination of leaves resulted in a decrease of $T_{1/2}$ by a factor of 2–5 within 10 min depending on light intensity. The results are summarized in Fig. 5. At light intensities of 100, 160, and 650 μmol m$^{-2}$ s$^{-1}$, $T_{1/2}^{ps}$ decreased in 10 min to 54, 40, and 17% of $T_{1/2}^{c}$, respectively (statistics with cells of $T_{1/2}^{c}$ $>2$ s; t test; $P < 0.05$; n=14, 15, 5 cells, respectively), i.e., higher light intensities induced significant increases in cell $Lp$.

In the results presented in Fig. 5, those cells were included, where decreases in turgor were up to 0.2 MPa during 10 min of illumination. Hence, the conclusion about significant effects of light and that they increase with increasing intensity is safe, because decreases in $T_{1/2}$ would have been even larger when keeping turgor strictly constant (see above). On the other hand, for cells having $T_{1/2}^{c}$ smaller than 2 s, the reduction in $T_{1/2}$ during 10 min of illumination was negligible and statistically different from other ratios at any high intensity (t test; $P < 0.05$; n=5 cells).

Discussion

For the first time, the data in this study show how the hydratics of intact corn leaves change at the cell level in response to changes in light intensity (from ambient light intensity of 40 μmol m$^{-2}$ s$^{-1}$ to increased light intensity) and how this interferes with transpiration (turgidity). The midrib parenchyma cells used were close to major vessels in its periphery and the midrib had stomata on both the adaxial and abaxial sides. Cells were located in the vicinity of photosynthetically active cells. Hence, they may be taken as a model system for the mesophyll cells of the leaf blade, which, as yet, cannot be used because they do not allow long-term measurements, as the cells from the midrib do. In an earlier study, Frensch and Schulze (1988) used the Bayreuth pressure probe, to measure turgor and water potential of individual leaf mesophyll and epidermis cells of Tradescantia virginiana. They investigated how this would change during illumination and transpiration. However, these authors were not able to measure changes of half-times of water exchange of cells,
T_{1/2}, and cell Lp because of problems with keeping those cells on the tip of the probe for a sufficiently long time period. However, upon illumination, they found responses in turgor, which were similar to those presented in this paper.

It may be argued that there was some scatter in the responses of cells when measuring T_{1/2} (Lp), and it took about 1 h to get fairly constant readings to start treatments. The reason for the noise is not known. It was not due to a leakiness of cells, since cells could be measured for up to 6 h at constant turgor. When turgor was kept constant at high values with the aid of the pressure chamber, stability improved and this suggested that local changes in the water status around a given cell may play a role (data not shown). It may be speculated that, when changing the apoplastic water content near a cell, this should also affect the local concentration of ions such as Ca^{2+} or pH, which may affect AQPs (Johansson et al., 1996, 1998; Tournaire-Roux et al., 2003; Alleva et al., 2006). It was clear that the noise was different from what was reported by Wan et al. (2004) for cortical cells of young corn roots. In root cells, there were often transient increases in T_{1/2} after puncturing, which then reduced to small values of T_{1/2}, which were stable for several hours. In the root, the transient changes had been referred to a mechanical gating of aquaporins (AQPs), namely, when large changes in water flow occurred such as during pressure relaxations at high peak values of turgor. Different from the root, the ‘noise’ observed in the leaf appeared to be stochastic. Most of it was circumvented by waiting for stable readings in T_{1/2} before starting treatments. However, despite the noise, the results showed that changes in T_{1/2} due to light and turgor were significant and effects real. Effects of light could be separated from those of turgor.

When separately measuring either the effects of light or turgor by keeping the other variable constant, it was clear that increasing light intensity reduced T_{1/2} and increased Lp. On the other hand, reducing turgor increased T_{1/2}. The result suggested that, in the intact transpiring plant, both effects interact with each other. For example, high light intensity may tend to intensify the water flow across living tissue, but, as soon as the water status (turgor/water potential) declines, cell Lp is reduced, most likely by a closure of AQPs. This seems to be a reasonable response tending to minimize water losses and to keep cells turgid, but there may be other variables in the system, such as the symplastic or apoplastic pH or pCa, which are known to affect AQP activity (Johansson et al., 1996, 1998; Tournaire-Roux et al., 2003; Alleva et al., 2006). It has been known that light changes the pumping of protons and Ca^{2+} (Shabala and Newman, 1999). With the set-up used in this paper, these variables could not be varied in a defined way. Excised leaves could be used to do this and to work out the role of the immediate ionic environment of protoplasts. Such experiments are currently being performed. The xylem is perfused with solutions of defined composition, which contain ABA (known to stabilize AQPs; Wan et al., 2004; Lee et al., 2005), or certain values of pH and pCa. It is also intended to use mercuric chloride, which is known to inhibit AQPs. Provided that AQPs were inhibited by light or turgor, there should be no effects of a treatment by HgCl_{2}. Otherwise, there should be an effect. Besides the gating of AQPs, it cannot be excluded at present that there are changes in expression of AQPs in response to light as well. In walnut, this seemed to happen within 30 min (Cochard et al., 2007).

At present, there is no direct proof that changes in cell Lp caused by turgor and light were really due to an action of AQPs as suggested at the end of the previous paragraph. However, it is difficult to see how changes in cell Lp of more than one order of magnitude could be provided by, for example, changes in the water permeability of the lipid bilayer or those of other transporters (Maurel, 1997; Tyerman et al., 1999). Closure of AQPs in intact plant cells, however, has shown reductions of Lp by as large as factors of between 4 and 20 (Henzler and Steudle, 1995; Zhang and Tyerman, 1999; Tyerman et al., 1999, 2002; Henzler et al., 2004; Ye and Steudle, 2006).

The present result that, at short T_{1/2} and high Lp, light could not affect Lp anymore, points in this direction. In this case, virtually all AQPs would have already been open prior to switching on the light.

The immediate effect of light tending to increase cell Lp found in this paper is in line with increases of K_{leaf} by light as found in HPFM studies (Sack et al., 2002; Nardini et al., 2005; Tyree et al., 2005). Increases of water permeability of parenchyma cells may contribute to increases in overall K_{leaf} as already suggested by these authors. However, before concluding this, the contribution of parenchyma cells to K_{leaf} should be fully sorted out. This information is still missing. Provided the parenchyma cells were one of the major components of K_{leaf}, the overall leaf hydraulics would have responded differently to light with the consideration of a decrease in turgor induced by light. In corn root cells, changes in turgor pressure have been suspected to affect water permeability via a gating of AQPs by what was called a ‘mechanical gating’ (Wan et al., 2004). The present results suggest that these effects were somewhat different. There was a different type of ‘mechanical action’ in the leaf cells in that absolute values of turgor rather than changes of turgor (and the corresponding water flows) were sensed. The decreased turgor induced by light caused an effect opposite to that of light, and this could have decreased K_{leaf}. In this case, it should again be stressed that this would only be valid when parenchyma cells play a substantial role in the overall leaf hydraulics. As in the present study, some caution is required before easily...
concluding about the effects of light on cell $L_p$ via changes of AQP activity from overall measurements of $K_{\text{leaf}}$, namely, with the issue related to turgor. It is hard to see how the effects of light and turgor could be separated during overall measurements.

The recent review by Sack and Holbrook (2006) summarized data on the effects of light on $K_{\text{leaf}}$ of 14 different herbaceous and woody species. The overall result was that $K_{\text{leaf}}$ strongly responded to light for many species. One may argue that the response of $K_{\text{leaf}}$ to light in corn may differ totally from that of other plants, i.e. not increasing with increasing light intensity. In response to light intensity, $K_{\text{leaf}}$ of corn should be measured. However, these measurements would require that turgor is kept constant to avoid interference by this variable. Experimentally this is not easy, but the problem should be solved in the future. The data at the leaf level, when available, should provide the direct linkage of hydraulicities between cell and whole leaf level. Sack and Holbrook (2006) expected diurnal changes of $K_{\text{leaf}}$ in such a way that $K_{\text{leaf}}$ should increase in response to increasing light and temperature. However, at high rates of transpiration $K_{\text{leaf}}$ should decline as the water potential and turgidity decreases. Using a steady-state evaporation technique, Brodribb and Holbrook (2006) reported that $K_{\text{leaf}}$ decreased in proportion to decreasing cell turgor in 16 out of the 19 species investigated. The authors suggested that hydraulic conductivity of living cells were affected by decreasing turgor, as shown here for cells of corn leaves. Responses of $K_{\text{leaf}}$ and cell $L_p$ to light should only be compared under the same experimental conditions including cell turgor. Otherwise, one may produce quite variable and, in part, contradictory results.

The high variability of cell $L_p$ measured could have originated not only by the temporal changes in cell $L_p$, but also by the inhomogeneity of cells in the tissue depending on their position. Water permeability of a parenchyma cell could vary between different cells in a tissue, which may be related to local effects of light, turgidity, or others (such as apoplastic concentrations of ions, see above). The present finding of a high variability of $T_{1/2}$ ($L_p$) is in line with the earlier observations of Westgate and Steudle (1985), who reported an inhomogeneous $L_p$ for the same tissue. According to these authors, the $L_p$ of individual cells varied by a factor of as large as 10 depending on the position. Perhaps, cells need to have an inhomogeneous $L_p$ depending on their location in the tissue. Different from the present paper, Westgate and Steudle (1985) could not make long-term measurements. However, using excised leaves, they did show that cell $T_{1/2}$ ($L_p$) was a crucial parameter determining the propagation of changes of water potential in the tissue in response to changes of pressure in the xylem, which affects the ability of tissue to dampen rapid changes of water potential (pressure) in the xylem. In that context, however, other parameters such as the elastic properties of cells and the hydraulic conductivity of the apoplast would have to be considered as well.

A variability of $T_{1/2}$ ($L_p$) of as large as a factor of 10 was found by Tomos et al. (1981) in leaf epidermal cells of Tradescantia virginiana, and by Zhu and Steudle (1991) in cortical cells of young corn roots (factor of 5 depending on cell layer). There was some variability in turgor of the soybean stem tissue cells (Nonami et al., 1987). There was also a variability of cell sizes and $e$ of up to a factor of 10 in leaf epidermal cells of Tradescantia virginiana (Tomos et al., 1981). Hence, there can be substantial variability in water relation parameters when measured at a microscopic scale. This would be evens out when measuring overall parameters (Scholander bomb, psychrometer, HPFM).

In the present study, the effects of relatively low intensities of light on cell $L_p$ compared to those in the field were investigated. To simulate field conditions, it should be further investigated how cell $L_p$ would respond to higher light intensities that are comparable to those in the field. At the relatively high light intensities of up to 2000 $\mu$mol m$^{-2}$ s$^{-1}$ encountered in the field, it is curious how living cells around the xylem will survive under high rates of transpiration (as they do). With very negative xylem pressure, cells may plasmolyse. There is convincing evidence that cells cannot sustain substantial negative turgor pressures, but up to now, there was no direct evidence by measurements of turgor of individual cells (Tyree, 1976; Oertli, 1986; Rhizopoloulou, 1997; Thürmer et al., 1999). It is curious to observe that, even at a light intensity which was lower than that in the field on a sunny day, cells experienced a turgor pressure that was lower than atmospheric. It is not yet clear how plant cells survive at high light intensities, i.e. how they improve their water status. Additional experiments are required to measure cell turgor and $T_{1/2}$ ($L_p$) under conditions of high rates of transpiration causing substantial tensions in the xylem. There may be an interplay of variable water supply from roots, stomata, turgor, and $L_p$ to orchestrate a water status that is favourable to the plant (Kramer and Boyer, 1995; Franks et al., 2007).

In conclusion, this detailed study of the hydraulics of parenchyma cells of leaves of intact plants, which are close to xylem vessels and stomata, revealed large ranges of water permeability (and $T_{1/2}$) of the cells. Despite some noise and variability in the hydraulics of cells, long-term measurements (up to 6 h for an individual cell) indicated that increasing light intensity increased cell $L_p$ (aquaporin activity). Turgor had an ameliorative effect as well. Using a root-pressure chamber, both effects could be separated from each other. Illuminating the leaves while keeping turgor constant increased cell $L_p$ by a factor of 3 within 30 min at a light intensity of 160 $\mu$mol m$^{-2}$ s$^{-1}$. Without keeping turgor constant, effects of light on cell $L_p$ was
compensated or even overcompensated by a decrease in turgor induced by an increase in transpiration. It is concluded that, without separating the effects of light and turgor, the results from measurements of overall leaf hydraulics ($K_{leaf}$) should be interpreted with caution. Although effects of light and turgor on cell $L_p$ are most likely related to a gating of AQPs, the mechanism(s) of the action of light and turgor are not yet clear.

**Acknowledgements**

The authors thank Mr Burkhard Stumpf (Department of Plant Ecology, Bayreuth University) for his expert technical assistance. They are indebted to Dr Carol A Peterson (Department of Biology, University of Waterloo, Ontario, Canada) and Dr Kosalana Rama-thungre (University of Bonn, Germany) for their help in interpreting leaf anatomy. This work was supported by a grant from the Deutscher Akademischer Austauschdienst, DAAD, to YXK.

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